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Lack of effect of D2 dopamine receptor TaqI A polymorphism on smoking cessation

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One previous report (Cinciripini et al., [2004] *Nicotine & Tobacco Research, 6*, 229–239) found that the D2 dopamine receptor (DRD2) TaqI A polymorphism was associated with smoking cessation: Carriers of the A1 allele were less likely to quit than were those who were not carriers. If confirmed, this finding would allow one to use precessation genotyping to predict the likelihood of successful quitting. The present study reports on results of a similar smoking cessation study and uses the same methods and data analysis in a larger number of smokers. It fails to replicate the effect of DRD2 TaqI A polymorphism on smoking cessation.

Introduction

In a recently published paper, Cinciripini et al. (2004) evaluated the relationship between smoking cessation treatment outcome and the DRD2 TaqI A restriction fragment length polymorphism in 134 smokers who took part in a clinical trial aimed to assess the efficacy of venlafaxine or placebo associated with brief counseling and nicotine replacement therapy. They demonstrated that smokers carrying the A1 allele of the DRD2 TaqI A polymorphism quit less often than did those who were not carriers of the A1 allele (OR=1.54, 95% CI=1.01–2.36). This finding corresponds well with the theory that DRD2 TaqI A polymorphism A1 carriers are more vulnerable to becoming drug dependent (Comings, Muhleman, Ahn, Gysin, & Flanagan, 1994); with the hypothesis that this polymorphism may play a role in tobacco dependence (Comings et al., 1996; Noble et al., 1994); and with the finding that the presence of the A1 allele may be associated with reduced dopamine synthesis, release, or DRD2 density (Jonsson et al., 1996, 1999). However, another randomized, placebo-controlled smoking cessation study with bupropion reported that the main effect of the DRD2 TaqI A genotype (as well as SLC6A3 dopamine transporter genotype) on abstinence rate was not significant at either time point of the smoking cessation study and was independent of the treatment effect (bupropion vs. placebo; Lerman et al., 2003).

These controversial results led us to analyze data of an unpublished randomized, placebo-controlled drug trial. We used the same data analysis and presentation as in Cinciripini et al. (2004) to allow maximum comparability of the results.

Method

Clinical study

Participants were 600 smokers recruited through newspaper, radio, and television advertisements to participate in a randomized, double-blind, multicenter, therapeutic trial of smoking cessation comparing three doses of a reversible monoamine oxidase A inhibitor, befloxatone, to placebo. All patients included had signed an informed consent for genotyping assessment. The participating centers' institutional review boards approved the study protocol. The trial consisted of a 3-month treatment period and a follow-up at 6 months. The treatments had no effect on abstinence rate. All participants

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received standardized counseling according to the National Cancer Institute recommendations. The main outcome measure was sustained abstinence during the last 4 weeks of the treatment period based on self-report and an expired-air carbon monoxide level of 10 ppm or less. All participants had been smoking 15 cigarettes/day or more over the past year. Some 52% of the sample was female, 85% was White, 8.8% was Black, 3% was Hispanic, and 2% was Asian American. Mean participant age was 43.7 years (SD=11.3), mean body mass index was 26.8 kg/m^2 (SD=5.3), and mean Fagerström Test for Nicotine Dependence (FTND) score was 5.6 (SD=2.1). After randomization, the participants attended weekly visits to assess abstinence status. The target quit date should have been between day 0 and day 10.

Genotyping

DNA was extracted from peripheral blood samples. The DRD2 Taq1 A polymorphism was determined by a standard PCR-RFLP assay (Grandy, Zhang, & Civelli, 1993) at the National Cancer Institute, in Bethesda, Maryland.

Data analyses

Associations between the demographic and diagnostic characteristics at baseline vs. genotype and the main outcome measure (sustained abstinence) were evaluated using chi-square tests for independence when the baseline characteristic was a categorical variable (gender, ethnicity, number of cigarettes smoked per day) and two-sample tests when the variable was continuous (age, FTND, Beck Depression Inventory [BDI] score at baseline, change in total withdrawal score between day 0 and day 7 and between day 0 and day 14, and change in desire to smoke between day 0 and day 7 and between day 0 and day 14).

Weekly point prevalence abstinence status was modeled as a function of genotype, treatment time of assessment (postquit weeks 1–9, 11, 13, 19, and 23), and their interactions: Genotype × treatment, genotype × time, time × treatment, and genotype × time × treatment. Time was treated as a categorical variable in the model. The site was treated as a random variable, and the subject was random effect nested within site.

To assess the effect of selected covariates on the inference about genotype, the model above was adjusted for all covariates of interest by including their main effects such as gender, ethnicity, number of cigarettes smoked per day at baseline, and BDI score at baseline. A backward elimination procedure starting with the three-way interaction and obeying the hierarchical principle was used to select the final model. We used the GLMMIX macro in SAS to estimate and test the model. Fit statistics (Akaike's information criterion; see Wolfinger, 1993) indicated that the unstructured banded model provided the best fit to the correlation structure for both models with and without including additional covariates. The general Satterthwaite approximation was used to calculate the denominator degrees of freedom for F tests.

Results

Out of 600 subjects, five had no information about their genotypes. We therefore deleted these data and analyzed data for the remaining 595 subjects. Table 1 shows allele frequencies. The frequency of the genotypes were in Hardy–Weinberg equilibrium, $\chi^2(2)=0.2014$, $p=.9042$. DRD2 Taq1 A polymorphism A1 frequency was somewhat lower (20.6% vs. 26%) and A2 allele frequency was somewhat higher (79.4% vs. 74%) than that found by Cinciripini et al. (2004). The frequency of the A1/A1 genotype was lower and closer to the values found by Lerman et al. (2003) and Berlin et al. (2000) (6.7% and 6.9%, respectively). In contrast to Cinciripini et al. (2004), in the univariate analysis a significant ethnic difference in the frequency of alleles was found (A1 carriers: White, 80%; Black, 11.4%; Hispanic, 4.1%; Asian, 3.7%; other, 1.2%; and for non-A1 carriers: White, 89%; Black, 6.6%; Hispanic, 2%; Asian; 0.9%; other, 1%; $p=.01$). No significant difference was found between A1 carriers and non-A1 carriers for gender, age, FTND score, number of cigarettes per day, or BDI score. Analysis of the main outcome measure of the clinical study (sustained abstinence) showed no significant difference between A1 carriers and non-A1 carriers (OR=0.74, 95% CI=0.46–1.18, $p=.207$). Similarly, we found no difference in end-of-treatment abstinence rates between A1 carriers and non-A1 carriers (OR=0.46, 95% CI=0.46–1.25, $p=.281$). A1 carriers had similar changes in total withdrawal symptoms and desire to smoke as did non-A1 carriers.

Figure 1 shows the weekly point prevalence abstinence rates by genotype at each visit. Inspection of

Table 1. DRD2 Taq I A polymorphism allele frequency and genotype.

Allele	Number of subjects	%
Taq1-A1	246	20.6
Taq1-A2	944	79.4
Total alleles	1,190	
A1/A1	36	6.1
A1/A2	210	35.3
A2/A2	349	58.7
Total number of subjects	595	

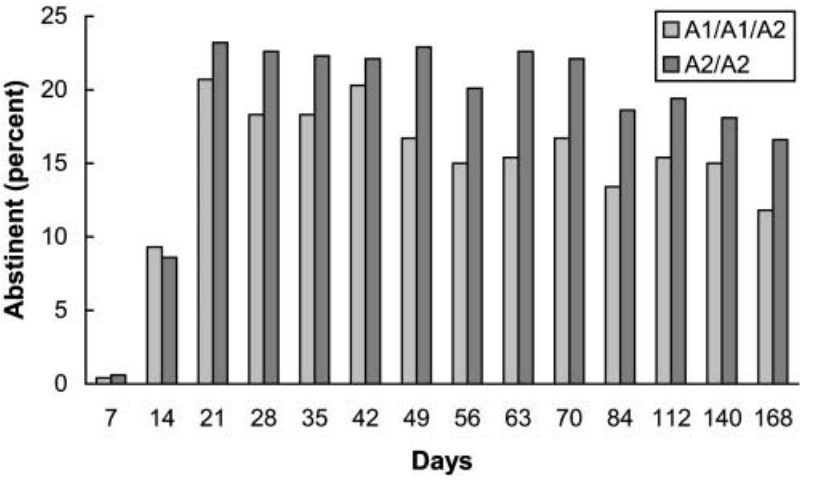

Figure 1. Weekly point prevalence abstinence by DRD2 genotype (Taq1 A polymorphism) (*OR*=0.725, 95% *CI*=0.5–1.05, *p*=.2, adjusted for all potential confounders).

this figure might suggest that point prevalence abstinence rates were lower in A1 carriers than in non-A1 carriers. However, based on the final model (Model A in Table 2), the effect of the genotype, $F(1, 527)=1.67$, $p=.20$, and that of treatment, $F(3, 521)=0.35$, $p=.79$, were not significant. The time effect was significant, $F(12, 394)=8.27$, $p<.001$.

When controlling for additional covariates, we found that the inference of genotype did not change (Model B in Table 2). Among the covariates, BDI score (<10 or ⩾10) was significantly associated with smoking cessation, $F(1, 515)=6.44$, $p=.01$. Smokers with BDI scores of 10 or more quit significantly less often than did those with scores of less than 10 (*OR*=2.57, 95% *CI*=1.24–5.35). None of the other covariates was a significant predictor of the point prevalence abstinence status of the study participants. In particular, no treatment effect occurred in any model ($p>.42$ in all three models). Because a significant ethnic difference was seen in the frequency of alleles, we reanalyzed the data for only Whites. The results were similar, and again, only the main effects of time and BDI score (<10 or ⩾10) were statistically significant (Model C in Table 2).

Discussion

The present study attempted to replicate the results of Cinciripini et al. (2004) in a greater number of smokers (595 vs. 134) who participated in a randomized, placebo-controlled, double-blind clinical drug trial. No main effect was found for DRD2 Taq1 A polymorphism on the main outcome measure (sustained abstinence rate). We tested further the effect of genotype, treatment, and time on weekly point prevalence abstinence rate by the mixed-effect model as Cinciripini et al. (2004) did.

Table 2. Full model results of fixed effects on weekly point prevalence abstinence rates.

Effect	Num*DF*	Den*DF*	*F* value	*p* value
Model A, All subjects (*N*=595)				
DRD2 Taq I A polymorphism	1	527	1.67	.20
Time	12	394	8.27	<.001
Model B, All subjects (*N*=595)				
DRD2 Taq I A polymorphism	1	517	2.47	.12
Time	12	395	8.22	<.001
BDI score	1	515	6.44	.01
Gender	1	526	0.30	.58
Cigarettes/day	2	520	2.84	.06
Ethnicity	4	523	0.72	.58
Model C, Whites only (*n*=508)				
DRD2 Taq I A polymorphism	1	439	2.30	.13
Time	12	337	7.08	<.001
BDI score	1	439	7.28	.01
Gender	1	445	3.06	.08
Cigarettes/day	2	446	2.73	.07

Note. BDI, Beck Depression Inventory; Den*DF*, denominator degrees of freedom; Num*DF*, numerator degrees of freedom.

Both models used the unstructured banded correlation within subjects with respect to the point abstinence over time. This analysis showed that point abstinence rates were not different between the two genotypes.

The present study had sufficient power to accept the hypothesis that DRD2 Taq1 A polymorphism has no clinically meaningful effect on smoking cessation. With 595 subjects, 246 A1 carriers and 349 non-A1 carriers, we had a 89% power to detect, with two-sided test and at $\alpha = .05$, the effect of genotype on point prevalence abstinence rates that corresponds to an odds ratio of 1.4. This value is below the odds ratio of 1.54 observed by Cinciripini et al. (2004). The power calculation was based on the mixed model with only main effects of genotype, treatment, and time. The estimated coefficients for time, treatment, and correlation structure within subjects in the present study were used for the calculation. Thus, although we used the same statistical method but with a bigger sample (595 subjects), we did not detect a significant association between the DRD2 Taq1 A polymorphism and smoking cessation. This finding is in line with Lerman et al. (2003), which found no main effect of DRD2 Taq1 A polymorphism on smoking cessation and which suggests instead a positive interaction effect of DRD2 Taq1 A polymorphism and dopamine transporter polymorphism.

Compared with Cinciripini et al. (2004), we observed a lower abstinence rate. All smokers in the Cinciripini et al. study received nicotine replacement therapy, whereas none did so in the present study, which could explain the difference in abstinence rates between the two studies.

If the present study had been able to replicate the results from Cinciripini et al. (2004), this would have suggested that determination of DRD2 Taq1 A polymorphism could have been used to predict the likelihood of successful smoking cessation. Unfortunately, the present study failed to confirm the results of Cinciripini et al. (2004). This means that precessation genotyping of DRD2 Taq1 A polymorphism to predict the likelihood of successful quitting is not useful in clinical practice.

DRD2 genotype determination, however, may be of interest in predicting response to nicotine replacement therapy. According to a recent study, effectiveness of nicotine patches was higher in women with the variant T (CT or TT) allele of the DRD2 compared with those with the more common CC genotype (Yudkin et al., 2004). Thus determination of the DRD2 polymorphisms in interaction with nicotine replacement therapies may predict efficacy in a subgroup of responders. Participants of the present study did not receive nicotine replacement therapy. Thus the discrepancy between results from Cinciripini et al. (2004) and the present study may come from a potential genotype $\times$ nicotine replacement therapy interaction.

In conclusion, the present study failed to replicate the effect of DRD2 Taq I A polymorphism on smoking cessation in smokers not treated with nicotine replacement therapy and shown in a previous report, despite similar study design, data analysis, and larger sample size.

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